Fetal Development in Cattle with Multiple Ovulations[1]

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ABSTRACT: Treatment of lactating and nonlactating parous cows (n = 379) with 12 mg of FSH-P to evaluate development of multiple bovine fetuses resulted in ovulation rates ranging from 1 to 27 corpora lutea (CL). Fertilization rate (i.e., ova fertilized at 6 to 8 d postmating, 80.0%) was not affected by ovulation rate. The percentage of fetuses developing normally at 51 to 53 d postmating decreased ($P < .01$) as ovulation rate increased; 1 CL, 100.0%; 2 CL, 100.0%; 3 CL, 66.7%; 4 CL, 45.8%; 5 CL, 33.3%; 6 to 10 CL, 13.6%; and > 10 CL, 8.9%. Of the 86 cows permitted to calve, 47 produced singles, 22 twins, 9 triplets, 7 quadruplets, and 1 quintuplets. Calf birth weight and gestational length decreased ($P < .01$) as the number of calves born increased from one to two to three. Smaller decreases ($P < .05$) in birth weight occurred among triplets, quadruplets, and quintuplets, whereas gestational length did not differ ($P > .1$) among these groups. Systemic progesterone concentrations in the dam were proportional ($P < .01$) to the number of fetuses in utero between d 126 and 266 for dams gestating one, two, or three or more fetuses; estrone sulfate was lower ($P < .01$) in dams with one than in those with two or more fetuses. Placental weight (i.e., cotyledons plus intercotyledonary membranes) per fetus at 52 ± 1 d of gestation and at term decreased as the number of fetuses increased. The chorioallantoic membranes were often fused among multiple fetuses and contained either all viable or all dead fetuses, but not both, within the same anastomosed placental unit. These results suggest that ovulation rate is the first limiting factor to increasing cow productivity for beef cattle because some bovine females had the capacity to gestate up to three fetuses per uterine horn, or a total of five fetuses, above which pregnancy was terminated.

Key Words: Multiple Births, Embryo Mortality, FSH, Estrone Sulfate, Progesterone, Cattle

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Introduction

The frequency of twin births in cattle is low (i.e., .5 to 6% of the births) and attempts to increase twinning with gonadotropin treatments have produced highly variable results (Bellows and Short, 1972). An assessment of the relationship between ovulation rate and twinning rate in Holstein cattle indicated that the frequency of twin ovulations was 13.1 vs 1.9% for twin births, suggesting a high incidence of fertilization failure and(or) embryonic mortality with multiple ovulations (Kidder et al., 1952). However, data obtained from cattle selected for twinning (Echternkamp et al., 1990) or from commercial slaughter plants (Hanrahan, 1983) suggested that approximately 50% of the twin ovulations resulted in twin births. Embryonic losses in ewes having multiple ovulations were 28 to 48% (Kelly and Knight, 1979). Pregnancy rates were also found to be approximately 50% lower in cattle with multiple ovulations than in those with single ovulations (Kidder et al., 1952).

Evaluations of cause(s) and time of reproductive losses in cows with multiple ovulations and fetuses have been limited to superovulated cows. Fertilization rate was reduced at 3 d after mating in FSH-treated cows with six or more vs less than six ovulation points (Bellows and Short, 1972). Although degenerate embryos were found in some

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FSH-treated cows, d 3 of gestation may have been too early to evaluate the influence of multiple embryos on early embryonic mortality (Maurer and Chenault, 1983). The objectives of the present study were to determine the effects of ovulation rate and(or) fetal numbers in utero on conception rate, embryonic survival and development, maternal progesterone and estrone sulfate (E_1SO_4) concentrations, and placental function and to assess uterine capacity in cattle.

Materials and Methods

Experiment 1. To produce multiple ovulations and subsequent births, 283 cyclic, parous Simmental-crossbred cows were given twice daily (a.m. and p.m.) injections of FSH (**FSH-P**; Burns Biotech, Omaha, NE; total dosage = 12 mg) for 4 d (2 mg of FSH × 2 for 2 d, and 1 mg × 2 for 2 d) plus 35 mg of prostaglandin $F_2\alpha$ (**PGF**; Lutalyse; Upjohn, Kalamazoo, MI) on the morning of the 4th d. The FSH treatment was initiated during the midluteal phase of the estrous cycle, which was accomplished by dividing the animals into two treatment groups and treating the groups 2 wk apart (November 19 to 22 and December 2 to 5). Cows were pastured with fertile Gelbvieh or Gelbvieh × Simmental bulls (10:1 cow:bull ratio) for 5 d subsequent to the last FSH injection with approximately 60 cows per pasture. Pregnancy rate and fetal numbers were assessed by ultrasonography at 45 to 60 d after mating; nonpregnant cows were culled from the experiment.

At approximately 100 d of gestation, cows were transferred from pasture to two open-front buildings equipped with Calan-Broadbent electronic head gates to enable measurement of feed consumption for individual animals. Animals were housed in groups of four animals of similar body size and fetal numbers within a pen. A diet composed (DM basis) of 71.4% corn silage, 25.9% alfalfa haylage, .5% dry shelled corn, and 2.2% vitamins and mineral supplementation in soybean meal was fed individually to achieve an ADG of .5 kg/d times the number of fetuses gestated. Body weight was measured at 28-d intervals. Two blood samples (10 mL) were collected from the tail vein by venipuncture 12 h apart at 28-d intervals. Blood was collected into heparinized syringes, stored immediately on ice, and centrifuged after each sampling period; plasma was collected and stored at −20° C until it was assayed for progesterone and E_1SO_4 by RIA. At parturition, calves were identified and weighed; the placenta (i.e., cotyledons plus intercotyledonary membranes) was collected and frozen from only those cows observed at parturition. Subsequently, each placenta was thawed, the cotyledons were dissected from the chorioallantoic membranes, and the cotyledons and intercotyledonary membranes were weighed separately, dried at 70° C for 60 h, and reweighed.

Concentrations of E_1SO_4 in maternal plasma were measured by the RIA procedure described by Eley et al. (1981). Free estrogens were extracted from duplicate 1-mL aliquots of plasma with 5 mL of diethyl ether and discarded. Subsequently, the plasma was incubated with 100 units of sulfatase enzyme at 37° C for 4 h. Liberated estrone was extracted with diethyl ether, dried under N_2, resuspended in .1% gel, .1 *M* PBS, pH 7.0, and quantified by a single-antibody, dextran-charcoal RIA. Estrone sulfate concentrations (nanograms/milliliter) were expressed as 1.4 times the assayed estrone concentration. Production and characterization of the estrone antiserum (WII BARC #4) have been described previously by Guthrie and Deaver (1979). The recovery of 10, 40, or 80 pg of E_1SO_4 from three volumes of plasma from a nonpregnant cow ranged from 93 to 105%. The inter- and intraassay CV in seven assays were 8.7 and 10%, respectively.

Progesterone was measured in duplicate 100-µL aliquots of plasma by the RIA procedure described by Echternkamp and Lunstra (1984). Two reference plasma samples included in each assay had mean concentrations and interassay CV of 1.2 ng/mL and 11.3% and 9.0 ng/mL and 12.6%, respectively. The intraassay CV was 7.5%.

Experiment 2. To assess the effect of ovulation rate on fertilization rate and early embryonic development, 154 nonlactating cows received the FSH (total dosage = 12 mg) and PGF treatments described for Exp. 1. Subsequent to the last FSH injection, cows were penned with fertile bulls for 2 h twice daily (a.m. and p.m.) for 5 d. Cows were monitored for estrus and mating times were recorded. Mated cows were slaughtered on either d 6 to 8 (n = 25) or d 51 to 53 (n = 71) after mating; reproductive tracts were collected and stored on ice immediately and transported to the laboratory for anatomical and pregnancy evaluation (Maurer and Echternkamp, 1985). Oocytes and embryos were flushed from both oviducts and uterine horns of cows slaughtered on d 6 to 8 after estrus using 30 mL × 2 of saline (.9 g of NaCl/100 mL of H_2O) per side. Flushings were searched microscopically (15×); identified oocytes and embryos were examined microscopically (75×) to assess fertilization (cleavage, number of polar bodies, and spermatozoa in zona pellucida) and(or) morphological development and viability of the embryos (stage of cleavage for gestational age, size and shape of blastomeres, and pyknotic cells). Attached embryos (d 51 to 53) were dissected from the uterine

Table 1. Induction of multiple births in cattle with follicle-stimulating hormone (Exp. 1)

Lactational status	No. of cows	Total conception	Type of pregnancy[a]				
			Single	Twin	Triplet	Quadruplet	Fetal degeneration[b]
Lactating	189	94 (49.7%)	35 (18.5%)	18 (9.5%)	11 (5.8%)	1 (.5%)	29 (15.3%)
Nonlactating	94	44 (46.8%)	17 (18.1%)	11 (11.7%)	6 (6.4%)	1 (1.1%)	9 (9.6%)
Overall	283	138 (48.8%)	52 (18.4%)	29 (10.2%)	17 (6.0%)	2 (.7%)	38 (13.4%)

[a]Fetal status was evaluated at 45 to 60 d postmating by ultrasonography.
[b]Uterus contained no viable fetuses and a predominance of ≥ 3 degenerate fetuses.

horns and examined anatomically for stage of development (development of fetal structures, body weight, and crown-rump length) and viability (clearness of amniotic fluid, firmness and coloration of fetus, and size for gestational age). Measurements of amniotic and allantoic fluid volume, fetal weight, crown-rump length, abdominal width, and wet and dry placental weight (i.e., cotyledons plus intercotyledonary membranes) were recorded for individual fetuses. Corpora lutea (CL) were dissected from the ovaries, and number and weight of individual CL were recorded.

Statistical Analyses. In Exp. 1, data were analyzed by ANOVA using the GLM procedure of SAS (1989). Data for gestational length, calf survival, and placental traits were analyzed by one-way ANOVA with number of calves produced as the main effect. The model for analysis of calf birth weights included calf number as the main plot and phenotypic sex of calf and the interaction of calf number × sex as the subplot; cow within calf number was used to test the main plot and the residual error to test the subplot. Comparisons between specific means were made by the lsd test. Small numbers of observations and(or) missing subgroups prevented evaluation of the effects of male:female sex ratio within a pregnancy on in utero fetal development. For the same reasons, data for freemartin and intact female calves were combined because freemartin and intact females were very similar phenotypically at birth. Diagnosis of the freemartin syndrome was based on anatomical characteristics of the reproductive tract either by visual examination at necropsy or by rectal palpation at 6 mo of age. Birth weight data were analyzed both with and without gestational length as a covariate.

For analysis of the hormone data, gestation was divided into seven 28-d gestational time periods with a range of ± 14 d: 126, 154, 182, 210, 238, 266, and 294 d. Progesterone and E_1SO_4 concentrations were analyzed by split-plot ANOVA using the GLM procedure (SAS, 1989) with fetal number as the main plot and gestational period and fetal number × gestational period as the subplot; cow within fetal number was the error term to test the main effect. The number of fetuses gestated was estimated from the number of calves delivered. Data for the cow with quintuplets were excluded from this analysis. Relationships among gestational length, placental measurements, calf birth weight, and hormonal concentrations were assessed by calculation of Pearson correlation coefficients within and among calf number groups (one, two, three, or four calves).

In Exp. 2, the effect of ovulation rate on fertilization rate and percentage of embryos normal at d 6 to 8 of gestation was determined by chi-square analysis. At d 51 to 53 of gestation, ovulation rate was categorized into seven groups: 1, 2, 3, 4, 5, 6 to 10, and > 10 CL. The total number of fetuses or the number of viable fetuses within the uterus was categorized as one, two, three, four, and five or greater. Main effect of ovulation rate on CL weight or of fetal number on individual fetal size, placental weight, and amniotic fluid volume were determined by ANOVA using the GLM procedure (SAS, 1989); cow within treatment was the error term to test main effect. Data for total placental weight and allantoic fluid volume were analyzed by one-way ANOVA with and without gestational length as a covariate. Because dead fetuses were not found among groups with one or two fetuses, data for viable and dead fetuses were analyzed separately. Differences between viable and dead fetuses were assessed by ANOVA on fetal and placental measurements adjusted to the respective means for triplet fetuses; cow within viability was the error term used.

Results

Experiment 1. Pregnancy rate for all cows treated with FSH in Exp. 1 was 48.8% (Table 1); pregnancy rate for cows observed in estrus was 60.4%. In addition to the 38 cows with only degenerate fetuses (Table 1), which were subsequently diagnosed to be nonpregnant, one set of twins, five sets of triplets, and one set of quadruplets were aborted between 165 and 244 d of

Table 2. Effect of birth numbers on gestational length and calf birth weight and survival rate[a]

Type of birth	No. of cows	Gestational length, d	No. of calves	Birth wt, kg	Survival rate[b]
Single	47	291.8 ± 1.0[d]	47	44.1 ± .8[d]	.98 ± .02[d]
Twin	22	284.3 ± 1.5[e]	44	34.8 ± .8[e]	.93 ± .02[d]
Triplet	9	273.9 ± 2.3[f]	26[c]	25.0 ± 1.0[fh]	.74 ± .03[eh]
Quadruplet	7	272.9 ± 2.6[f]	27[c]	21.8 ± 1.0[i]	.64 ± .03[i]
Quintuplet	1	274.0	5	16.6 ± 2.4[gj]	1.00 ± .00[d]

[a]Least squares means ± SE. Data for cows aborting twin (n = 1), triplet (n = 5), or quadruplet (n = 1) fetuses between 165 and 244 d of gestation were excluded from the analysis.
[b]Calf survival at 24 h after parturition.
[c]Birth weight of a mummified fetus was excluded.
[d,e,f,g]Means within a column without a common superscript differ ($P < .01$).
[h,i,j]Means within a column without a common superscript differ ($P < .05$).

gestation. An additional seven dams diagnosed (by ultrasonography) as pregnant with multiple fetuses did not produce a calf. Length of gestation and calf birth weight and survival (Table 2) decreased ($P < .01$) as the number of calves gestated increased; differences between triplets, quadruplets, and quintuplets were not significant ($P > .10$) for gestational length. Birth weight of an individual male or female calf (Table 3) decreased by approximately 25% for each increase in calf number from single to twin to triplet. The reduction in birth weight was less for quadruplets and quintuplets (i.e., 10 to 15%). Male calves were heavier ($P < .01$) than contemporary female calves (34.6 ± .7 vs 32.1 ± .6 kg); freemartin calves (i.e., 9 twin, 10 triplet, and 6 quadruplet freemartin calves) were classified as intact females in the analysis. The calf number × sex of calf interaction was not significant ($P > .10$) for birth weight. Although gestational length had a positive effect ($P < .01$) on birth weight, inclusion of gestational length as a covariate in the analysis did not remove the effect of calf number ($P < .01$) or sex ($P < .01$) on birth weight (Table 3). Calf birth weight increased by .35 kg/d of gestational length. The incidence of calf mortality at or within 24 h after parturition (Table 2) was increased ($P < .01$) for triplet and quadruplet calves, as was the incidence of dystocia associated with abnormal presentation of the calves in cows producing multiple births. The number of abnormal presentations for cows producing twins, triplets, quadruplets, and quintuplets was 11 of 22, 3 of 9, 6 of 7, and 1 of 1, respectively, compared with 3 of 47 for cows producing a single calf.

Under close observation, a complete placenta was obtained from 17 cows producing a single calf, 11 producing twins, 6 producing triplets, and 2 producing quadruplets (Table 4); unobserved calving or prolonged retention prevented collection of the placenta from the other cows. The effect of calf number on total weight of wet or dry cotyledons ($P < .05$), of wet or dry intercotyledonary membranes ($P < .01$), or of wet ($P < .05$) or dry ($P < .01$) total placenta (cotyledons plus membranes) resulted from an approximately twofold increase in placental weight for cows delivering four calves. Dry weight of membranes ($P < .01$) and total placenta ($P < .05$) also differed for one vs three calves. Generally, there was a trend for placental, especially membranal, weight to increase with number of calves. Number of cotyledons within a placenta did not differ ($P > .10$) among calf groups. Total placental weight (dry) tended ($P < .10$) to increase with gestational length.

Table 3. Effect of birth numbers and sex of calf on calf birth weight

Type of birth and sex	No. of calves	Unadjusted birth wt, kg	Adjusted birth wt, kg[a]
Single			
Male	19	46.5 ± .8[c]	43.0 ± .8[c]
Female	28	42.4 ± .7[d]	38.7 ± .8[d]
Twin			
Male	21	35.6 ± .8[e]	34.4 ± .8[e]
Female	23	33.8 ± .7[e]	33.9 ± .8[e]
Triplet[b]			
Male	16	26.0 ± .9[fh]	30.0 ± .9[fh]
Female	10	22.6 ± 1.1[fgi]	27.0 ± 1.0[fi]
Quadruplet[b]			
Male	10	22.8 ± 1.1[fgi]	27.9 ± 1.0[f]
Female	17	21.2 ± .9[gi]	23.2 ± .9[gj]
Quintuplet			
Male	2	16.4 ± 2.5[gj]	18.5 ± 2.5[gk]
Female	3	16.7 ± 2.1[gj]	18.5 ± 2.1[gk]

[a]Data for calf birth weight were analyzed with gestational length ($P < .001$) as a covariate.
[b]Data for a mummified fetus were excluded from the analyses.
[c,d,e,f,g]Means within a column without a common superscript differ ($P < .01$).
[h,i,j,k]Means within a column without a common superscript differ ($P < .05$).

Table 4. Effect of birth numbers on placental traits at parturition[a]

Variable	No. of calves born[b]			
	One	Two	Three	Four
No. of cows	17	11	6	2
No. of cotyledons	70.2 ± 6.2	66.1 ± 7.7	57.5 ± 10.3	88.5 ± 18.0
Cotyledonary wt, g				
Wet	1,440.4 ± 128.8[c]	1,372.2 ± 160.1[c]	1,069.0 ± 216.8[e]	2,330.8 ± 375.4[df]
Dry	153.6 ± 14.4[c]	148.1 ± 17.9[c]	125.3 ± 24.3[e]	269.5 ± 42.0[df]
Membranal wt, g				
Wet	1,510.4 ± 133.9[ce]	1,755.6 ± 166.4[c]	2,091.2 ± 225.3[d]	2,828.5 ± 390.2[df]
Dry	156.5 ± 14.2[eg]	184.1 ± 17.7[g]	243.0 ± 24.0[cf]	353.6 ± 41.5[dfh]
Total placental wt, g				
Wet	2,950.8 ± 248.4[e]	3,127.7 ± 308.8[c]	3,160.1 ± 418.1[c]	5,159.3 ± 724.1[df]
Dry	310.2 ± 27.1[g]	332.3 ± 33.7[e]	369.3 ± 45.6[e]	623.1 ± 79.0[fh]

[a]A complete placental unit was obtained from only a portion of the cows calving.
[b]Number of calves born to a cow at the observed calving.
[c,d]Means within a row differ among birth groups ($P < .05$).
[e,f]Means within a row differ among birth groups ($P < .01$).
[g,h]Means within a row differ among birth groups ($P < .001$).

Concentrations of progesterone in the maternal circulation (Figure 1) were affected ($P < .01$) by both the number of fetuses gestated (i.e., number of calves born) and stage of gestation; the fetal number × gestational period interaction was significant ($P < .01$) also. Progesterone concentrations between 126 and 238 d of gestation were related ($P < .01$) to the number of fetuses in utero for cows gestating one, two, or three fetuses (Figure 1), whereas concentrations did not differ between cows gestating three vs four fetuses, except at 210 d ($P < .01$). At 266 d of gestation, progesterone was higher in cows gestating two or three fetuses than in cows with one ($P < .05$) or four ($P < .01$) fetuses and was higher ($P < .05$) in cows with one than in cows with four fetuses.

Variation in systemic progesterone concentrations among gestational periods included a temporal increase ($P < .01$) at 154 d (i.e., relative to 126, 182, or 266 d) for all four fetal groups. A second elevation in progesterone concentrations occurred at 210 and 238 d in cows gestating one ($P < .01$) or two ($P < .03$) fetuses and at 210 d for cows with three ($P < .01$) fetuses. In cows gestating three or four fetuses, progesterone declined significantly ($P < .01$) from 238 to 266 d; all the cows gestating four fetuses calved before 280 d of gestation. Maternal progesterone concentrations decreased ($P < .01$) precipitously during the last 28 d of gestation in all four fetal groups. Absence of an increase at 210 d of gestation and an earlier prepartum decrease (shorter gestational length) in progesterone in cows gestating four fetuses resulted in the significant ($P < .01$) group × time interaction.

The significant ($P < .01$) effect of fetal number on maternal E_1SO_4 concentrations resulted primarily from lower E_1SO_4 concentrations from 154 d of gestation to parturition for cows gestating one fetus (Figure 2). Concentrations of E_1SO_4 at 126 d of gestation did not differ ($P > .05$) among the four fetal groups. Systemic E_1SO_4 concentrations did not differ ($P > .05$) among cows gestating two, three, or four fetuses except for higher ($P < .01$) E_1SO_4 concentrations at 266 d of gestation for cows with four fetuses.

Estrone sulfate concentrations in the maternal circulation increased ($P < .01$) quadratically with duration of gestation; the gradual increase in E_1SO_4 between 126 and 238 d of gestation was followed by rapid increases between 238 and 266 d and between 266 and 294 d of gestation. Except between 182 and 210 d of gestation, E_1SO_4 concentrations increased significantly ($P < .01$) during each 28-d interval. Although the trend for E_1SO_4 to increase with gestational length was similar among the four fetal groups, group differences in magnitude ($P < .01$) of the increase in circulating concentration of E_1SO_4 resulted in a significant ($P < .01$) stage of gestation × number of fetuses interaction.

Use of maternal E_1SO_4 as a criterion to diagnose twin pregnancies was evaluated. The initial diagnostic criterion was those females in which maternal circulating concentrations of E_1SO_4 exceeded the period mean for single-bearing females by 50% at two or more of the seven gestational periods. Based on this criterion, 6 of 47 (12.8%) cows gestating a single fetus were diagnosed incorrectly as gestating twins and 8 of 22 (36.4%) cows with twins incorrectly as singles. Use of the same criterion for maternal progesterone concentrations did not identify any cows with multiple

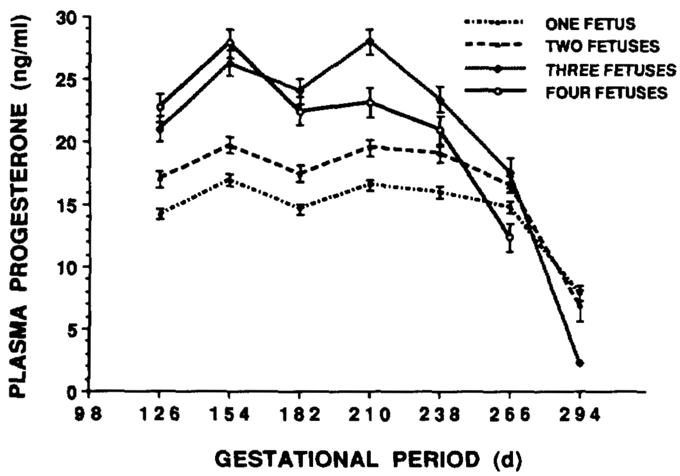

Figure 1. Relationship between number of fetuses in utero and maternal circulating concentrations of progesterone. Progesterone concentrations were proportional ($P < .01$) to number of fetuses in utero from 126 to 266 d of gestation except for three vs four fetuses ($P > .05$).

fetuses. Several other percentages of the mean for E_1SO_4 and progesterone were evaluated, but the accuracy for diagnosing twins did not improve.

When evaluated among calf groups, individual calf birth weight was correlated negatively with total birth weight ($P < .01$), with wet ($P < .05$) or dry ($P < .01$) intercotyledonary membranal weight, and with maternal concentrations of progesterone ($P < .01$) and E_1SO_4 ($P < .01$) from 126 to 238 d and from 126 to 266 d of gestation, respectively (Table 5). Conversely, total calf birth weight per dam was correlated positively with dry weight of the membranes ($P < .05$) and with maternal progesterone ($P < .01$) and E_1SO_4 ($P < .01$) concentrations from 126 to 238 d and from 126 to 294 d of gestation, respectively (Table 5). Likewise, a positive correlation ($P < .05$) was found between calf birth weight of singletons and maternal E_1SO_4 concentrations at 182 (r = .46), 266 (r = .47), and 294 (r = .55) d of gestation, whereas correlations for the other four gestational periods approached significance ($P < .10$). Maternal concentrations of progesterone and E_1SO_4 were correlated positively ($P < .05$) between 126 and 238 d of gestation (r = .21 to .31) among groups, whereas the positive correlations within calf number groups were small ($P > .10$).

Experiment 2. Distribution of ovulation rates and percentage of normal embryos, abnormal embryos, and unfertilized oocytes at 6 to 8 d postmating are reported in Table 6. Ovulation rate in cows slaughtered 6 to 8 d postmating ranged from one to five CL with a predominance of cows with one (48%) or two (40%) CL. Ovulation rate did not affect ($P > .05$) the percentage of embryos or oocytes classified as normal embryos, abnormal embryos, or unfertilized oocytes. An embryo or oocyte was not recovered from two cows with one CL, and only one oocyte was recovered from two cows with two CL.

Ovulation rate, measured at 51 to 53 d postmating, ranged from 1 to 27 (Table 7). Weight of the individual CL decreased as ovulation rate increased from one to two ($P < .05$) to three ($P < .01$), but individual weights were similar ($P > .10$) for ovulation rates ranging from 3 to 27. All fetuses recovered from females with one or two CL exhibited normal development (Table 8). When ovulation rate was three or greater, the number ($P < .05$) and percentage of dead (or degenerate) or unrecovered fetuses ($P < .01$) increased as ovulation rate increased (Table 8). The maximum number of viable fetuses located within a uterine horn on d 51 to 53 was three fetuses or within a uterus was five fetuses. When these numbers were exceeded, degenerate fetuses were found (e.g., two of seven cows with four CL had four degenerate fetuses located in one uterine horn, four of nine cows with five CL had four or five degenerate fetuses in one horn, etc.).

Weight, length, width, and amniotic fluid volume for fetuses viable at 51 to 53 d postmating (Table 9) were 6.87 ± .13 g, 44.2 ± .4 mm, 14.5 ± .2 mm, and 29.8 ± .9 mL, respectively, and were unaffected ($P > .10$) by number of fetuses, whether evaluated by number of viable fetuses or total number of fetuses within the uterus. Placental weight (dry) per fetus (Table 9) decreased ($P < .05$) as the number of fetuses per uterus increased. In

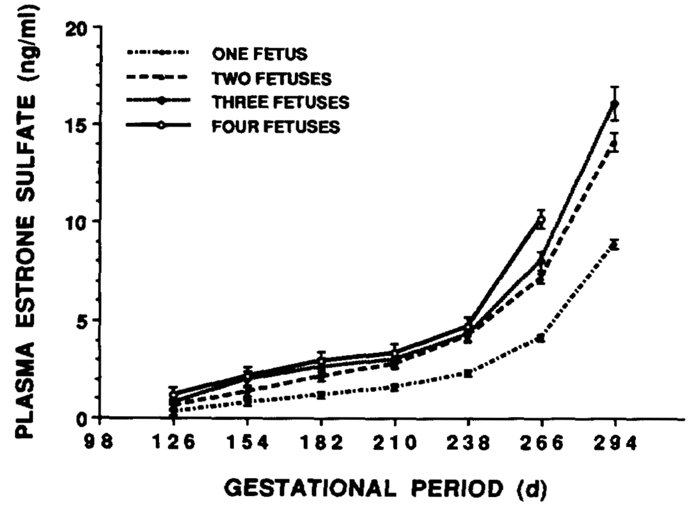

Figure 2. Relationship between number of fetuses in utero and maternal circulating concentrations of estrone sulfate. Estrone sulfate concentrations were lower ($P < .01$) throughout gestation in cows with single than in those with multiple fetuses and were proportional ($P < .01$) to number of fetuses at 266 d of gestation.

pregnancies of multiple fetuses, the chorions were connected physically among individual fetuses to form either one or two placental units with the attached placentas traversing through the uterine body into both uterine horns. As illustrated in Figures 3 and 4, fetuses classified as dead (or degenerate) were significantly ($P < .01$) smaller than the viable fetuses and had reduced ($P < .01$) placental weights (dry) and amniotic fluid volumes. Unlike size of viable fetuses, size of the dead fetuses decreased as number of fetuses in utero increased. As illustrated in Figure 5, viable and dead fetuses were not found in the same anastomosed placental unit. Death of one multiple bovine fetus seemed to result in death of all fetuses within the same anastomosed placenta. Total placental (dry) weight and total volumes of allantoic and amniotic fluid were greater ($P < .01$) in cows gestating two or more fetuses than in those gestating a single fetus.

Discussion

The major limitation to increased productivity in the beef cattle herd is the low level of productivity of the reproducing bovine female. Except for a few selected populations of cattle (Morris and Day, 1987; Echternkamp et al., 1990; Gregory et al., 1990a) most bovine females release only one oocyte per estrous cycle and, consequently, produce one progeny per year. Because the frequency of identical twins in cattle is very low, the low incidence of multiple ovulations in cattle is the first obstacle for increasing productivity in cattle (Echternkamp et al., 1990). The incidence of twin or multiple births has been increased in cattle by the administration of exogenous gonadotropins (e.g., pregnant mare's serum gonadotropin, **PMSG**, Turman et al., 1968; FSH, present study) or by the intrauterine addition of a second embryo (Anderson et al., 1979). Preliminary trials in our laboratory with the described FSH-P treatment resulted

Table 5. Correlations among calf birth weight, placental size, and maternal hormonal concentrations

Variable	Calf birth wt	Total calf wt	Total cotyledonary wt (dry)	Total membranal wt (dry)	Total placental wt (dry)[b]
Calf birth wt	—	−.43**	−.07	−.44**	−.29*
Total calf wt[a]	−.43**	—	.00	.34*	.21
Cotyledonary					
No.	.11	−.12	.69**	.40**	.57**
Wet wt	.03	−.05	.96**	.66**	.86**
Dry wt	−.07	.00	—	.74	.93**
Membranal wt					
Wet	−.36*	.21	.75**	.94**	.90**
Dry	−.44**	.34*	.74**	—	.94**
Total placental wt[b]					
Wet	−.21	.10	.91**	.86**	.95**
Dry	−.29*	.21	.93**	.94**	—
Progesterone					
P-1[c]	−.46**	.23*	.03	.29*	.16
P-2	−.43**	.24*	.03	.31*	.17
P-3	−.52**	.35**	.08	.33*	.26
P-4	−.50**	.32**	.08	.38**	.23
P-5	−.44**	.25*	−.11	.13	−.02
P-6	−.10	.03	−.05	.10	−.02
P-7	−.10	−.24	−.33	−.40	−.44
Estrone sulfate					
P-1	−.42**	.54**	.09	.30*	.21
P-2	−.52**	.63**	−.06	.31*	.12
P-3	−.53**	.66**	−.13	.30*	.07
P-4	−.47**	.65**	−.05	.17	.04
P-5	−.47**	.54**	.08	.29	.18
P-6	−.54**	.63**	.01	.21	.12
P-7	−.21	.52**	−.69*	.01	−.54

[a]Total weight of calves born per dam.
[b]Total weight of cotyledons plus intercotyledonary membranes.
[c]Gestational period.
*$P < .05$.
**$P < .01$.

Table 6. Effect of ovulation rate on fertility and embryonic survival at 7 (± 1) days postmating[a]

		No. and percentage of embryos or oocytes		
Ovulation rate[b]	No. of cows	Normal embryo	Abnormal embryo	Unfertilized oocyte
1 CL	12	7 (70.0)[c]	2 (20.0)	1 (10.0)
2 CL	10	9 (50.0)	3 (16.7)	6 (33.3)
3 CL	1	3 (100.0)	—	—
4 CL	1	4 (100.0)	—	—
5 CL	1	3 (60.0)	1 (20.0)	1 (20.0)

[a]Cows received follicle-stimulating hormone twice daily for 4 d (total dosage = 12 mg).
[b]Ovulation rate was the number of copora lutea (CL) present at 6, 7, or 8 d postmating.
[c]Percentage of embryos and oocytes recovered. An embryo or ovum was not recovered from two cows with one CL and a single embryo was recovered from two cows with two CL.

in ovulation rates of one, two, or three, but ovulation rates obtained in the present trial were more variable, ranging from 1 to 27, as reported by other investigators (Gordon et al., 1962; Bellows and Short, 1972). Likewise, variable success rates in producing multiple births in cattle have been experienced with embryo transfer (Diskin et al., 1987). Although calf birth weight and gestational length are reduced in cows gestating multiple fetuses (Turman et al., 1968; Anderson et al., 1979; Gregory et al., 1990b), our results suggest that the uterus of some bovine females is capable of gestating up to five fetuses, or three fetuses per uterine horn. Similarly, a maximum of five calves were produced by PMSG-treated Hereford × Angus cows (Turman et al., 1968). As reported by Bellows and Short (1972), an increase in ovulation rate did not have a detrimental effect on fertilization rate, or on the number of ovulations not accounted for (d 51 to 53) except in two cows with an ovulation rate of 10 and 17. When ovulation rate exceeded two, the frequencies of dead, degenerate fetuses (Exp. 2) and of first- and third-trimester abortions (Exp. 1) were increased. The stage of fetal development for most dead or degenerate fetuses suggested that death occurred at approximately d 35 of gestation. In addition, death of a fetus after anastomoses of the placentas seemed to result in death of all fetuses within the same fused placental unit. Of particular interest was the observation in Cow 812714 (Figure 5) that each uterine horn contained a separate placental unit; the left uterine horn contained three fetuses exhibiting normal fetal development within the same fused placental unit, whereas the fused placental unit in the right uterine horn contained three small, dead fetuses (approximately 35 d of development).

Based on the number and distribution of viable and dead fetuses found at 52 d of gestation (Exp. 2), it is hypothesized that the bovine uterus has a maximum capacity for fetal numbers. This capacity may vary among females as well as among genetic populations. If the number of fetuses developing at approximately 35 d of gestation exceeds this upper limit for uterine capacity, there is an inherent mechanism to terminate the pregnancy. In both Exp. 1 and 2, the maximum number of viable fetuses was five per uterus or three per uterine horn. Unlike the porcine uterus (Dziuk, 1985), the bovine uterus does not seem to have the capability to selectively resorb fetuses. This species difference in fetal selection may be linked to species differences in placental morphology and function. Based on the incidence of intersex mosaicism in cattle and swine (Vogt, 1968), it is postulated that the incidence of placental anastomosis is approximately 90% in cattle, compared with < 10% in swine. Because of the anastomosis of blood supplies among multiple bovine fetuses, either the bovine female may be unable to selectively kill and resorb individual fetuses or death of one fetus generates sufficient toxins to kill all of the fetuses with a common blood supply. Also, repeated evaluations of early fetal development by ultrasonography in cattle selected for natural twinning have revealed sequential death of some twin fetuses (unpublished data). The contribution of fetal death to the abortion of multiple fetuses in later stages of gestation was not evaluated.

The reduction in birth weight of individual calves associated with multiple births was not reflected in fetal weight at 52 d of gestation. Similarly, Bellows et al. (1990) found no difference in weight between single and multiple fetuses at 95 d of gestation, but single fetuses were heavier at 180 d of gestation. Because 80% of the fetal growth for a singleton bovine fetus occurs during the last 3 mo of gestation (Ferrell et al., 1976), it is

Table 7. Effect of ovulation rate on weight of corpora lutea (CL) at 51 to 53 days of gestation[a]

Ovulation rate[b]	No. of cows	No. of CL	CL wt, g
1 CL	16	16	5.28 ± .21[c]
2 CL	10	20	4.17 ± .19[d]
3 CL	6	18	2.65 ± .20[e]
4 CL	6	24	2.28 ± .19[e]
5 CL	9	45	2.15 ± .13[e]
6 – 10 CL	10	81	1.89 ± .09[e]
> 10 CL	3	56	2.22 ± .11[e]

[a]Cows received follicle-stimulating hormone twice daily for 4 d (total dosage = 12 mg).
[b]Number of CL per pair of ovaries.
[c,d,e]Means without a common superscript differ ($^{c,d}P < .05$; $^{d,e}P < .01$).

Table 8. Distribution of dams by ovulation rate and by fetal number and status

Fetal status and no. of CL[a]	No. of cows[b]	No. of fetuses							
		0	1	2	3	4	5	> 5	$\bar{x}$[c]
Normal									
1	16	—	16	—	—	—	—	—	1.0 ± .0
2	10	—	—	10	—	—	—	—	2.0 ± .0
3	6	1	2	—	3	—	—	—	1.8 ± .5
4	7	2	—	2	1	2	—	—	2.1 ± .5
5	9	4	—	3	—	1	1	—	1.7 ± .4
6 – 10	10	6	—	1	3	—	—	—	1.1 ± .4
> 10	3	1	—	1	1	—	—	—	1.7 ± .7
Degenerate									
3	6	5	—	—	1	—	—	—	.5 ± 1.8[e]
4	7	4	1	—	—	2[d]	—	—	1.3 ± 1.7[e]
5	9	3	—	2	—	1[d]	3[d]	—	2.6 ± 1.5[ef]
6 – 10	10	1	—	—	2	—	1	6[d]	5.9 ± 1.4[fg]
> 10	3	1	—	—	—	1	—	1[d]	10.3 ± 2.5[g]
Ovulation unaccounted for									
3	6	4	—	2	—	—	—	—	.7 ± .9[h]
4	7	4	2	1	—	—	—	—	.6 ± .9[h]
5	9	4	4	—	1	—	—	—	.8 ± .8[h]
6 – 10	10	7	—	2	—	—	—	1	1.1 ± .7[h]
> 10	3	1	—	—	—	—	1	1	6.3 ± 1.3[i]

[a]Number of corpora lutea dissected from a pair of ovaries.
[b]Total number of females.
[c]Mean (± SE) response based on total number of females.
[d]Number of fetuses per uterine horn was > 3 and normal fetuses were not present.
[e,f,g]Means within a category without a common superscript differ ($P < .05$).
[h,i]Means within a category without a common superscript differ ($P < .01$).

suggested that the lower birth weights of multiple calves result from decreased growth rates during the last trimester of gestation. Although fetal weight did not differ between single and multiple fetuses at 52 and 95 d of gestation, placental weight per fetus was lower for multiple fetuses at 52 and 95 d in the respective studies. Thus, the decreased fetal growth in utero for multiple fetuses may occur secondarily to an adverse effect of multiple fetuses on placental development, or because of insufficient placental mass and nutrient exchange. The contribution of physical constraint or metabolic stress, or both, to the increased incidence of last-trimester abortions or a shorter gestational length for multiple fetuses is unknown. Although a reduction in prenatal growth for multiple fetuses resulted in lighter birth weights, a similar trend for male calves to be heavier at birth continued to persist among multiple births. Again, these results suggest that genetic differences in prenatal growth between sexes are expressed earlier in gestation than the restrictive effect of fetal number (Bellows et al., 1990).

Table 9. Effect of number of viable fetuses gestated on fetal size, placental weight, and amniotic fluid volume at 52 days of gestation[a]

Fetal traits[b]	No. of viable fetuses per dam				
	1	2	3	4	5
No. of cows	17	18	7	3	1
No. of fetuses	17	36	21	12	5
Weight, g	6.57 ± .31	7.01 ± .21	7.03 ± .28	7.31 ± .37	7.13 ± .57
Length, mm	43.3 ± .3	44.4 ± .2	45.2 ± .3	45.7 ± .3	46.2 ± .5
Width, mm	14.5 ± .5	14.7 ± .3	14.2 ± .4	14.4 ± .6	14.9 ± .9
Placenta (dry), g	2.19 ± .11[c]	1.63 ± .08[df]	1.33 ± .1[g]	1.21 ± .14[eg]	1.21 ± .21[efg]
Amniotic volume, mL	28.1 ± 5.5	30.1 ± 3.8	32.3 ± 4.3	30.9 ± 6.6	39.3 ± 10.2

[a]Mean fetal age was 52.0 ± .2 d.
[b]Data are reported per fetus.
[c,d,e,f,g]Means without a common superscript differ ([c,d,e]$P < .01$; [f,g]$P < .05$).

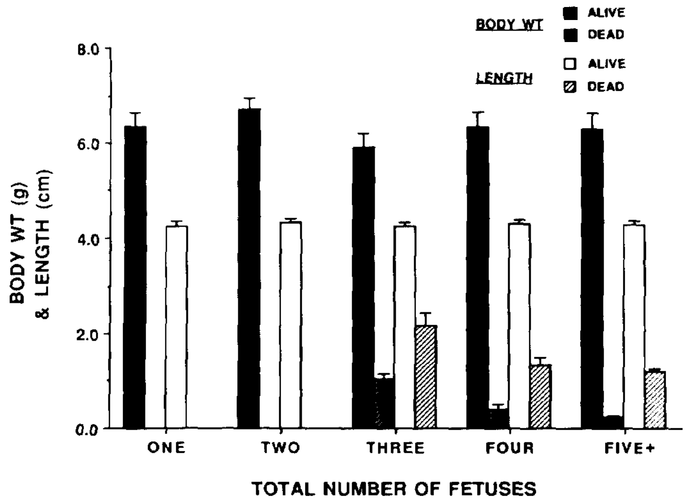

Figure 3. Comparison of fetal body weight and length for viable vs dead fetuses by total number of fetuses in utero at 51 to 53 d of gestation. Viable fetuses were heavier ($P < .01$) and larger ($P < .01$) than dead fetuses. Total number of fetuses did not affect ($P > .05$) the size of viable fetuses, but size of dead fetuses decreased ($P < .01$) with increased fetal number.

Missing combinations and limited numbers of observations in the nine sets of triplets, seven sets of quadruplets, and one set of quintuplets prevented evaluation of the effect of male:female sex ratio on intrauterine fetal development. Laster et al. (1971) reported that as the male:female sex ratio increased, the proportion of sex nodules decreased, and the degree of transformation of the female reproductive system increased. Diagnosis of the freemartin syndrome in the present studies was based on length of the vagina and on anatomical examination of the reproductive tract by rectal palpation. Because freemartin calves were similar phenotypically to intact female calves, data for freemartin and female calves were combined in the statistical analyses.

As the number of calves per dam increased, total calf birth weight increased, as did placental weight and circulating concentrations of progesterone and E_1SO_4 in the maternal blood, and, thus, significant positive correlations were measured among these four variables. Conversely, as the number of calves within a gestation increased, birth weight of individual calves decreased, resulting in negative correlations among calf birth weight, placental weight, and hormonal concentrations. Several investigators have reported a positive relationship between calf birth weight and placental weight or maternal E_1SO_4 concentrations, or both, in cows gestating a single calf (Thatcher et al., 1980; Head et al., 1981; Echternkamp, 1984). Collectively, these results suggest that size and functionality of the placenta influence growth and size of the calf and provide evidence for significant variation among animals for total placental size and function. These positive relationships between calf birth weight and placental size and function concur with reduced fetal and placental development in heat-stressed ewes (Alexander and Williams, 1971; Bell et al., 1989) or in ewes with a surgical reduction in caruncular area (Alexander, 1964). However, evidence exists for compensatory development of the fetal placentomes to maintain fetal development and steroidogenesis in undernourished cows (Rasby et al., 1990) and in ewes with caruncles surgically removed (Alexander, 1964). Cotyledonary weight was not correlated with total calf birth weight, but it is doubtful that a reliable estimate of cotyledonary size or area was obtained as the cotyledonary region of some placentas had undergone considerable necrosis by the time of expulsion, especially at the posterior end and in cows delivering multiple calves.

Concentrations of E_1SO_4 measured in the maternal circulation are presumably of placental origin and proportional to total surface area and(or) steroidogenic activity within the placentomes (Thatcher et al., 1980); thus, measured increases in maternal E_1SO_4 concentrations with fetal number and duration of gestation presumably reflect increases in placental size and(or) function. Contri-

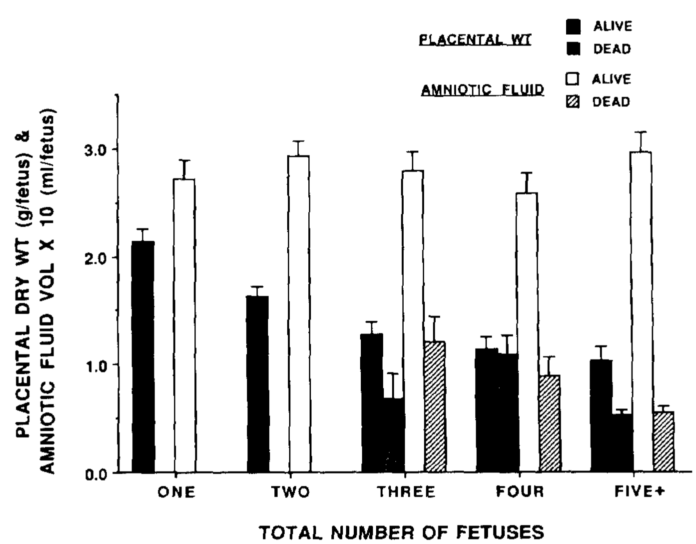

Figure 4. Comparison of placental weight (dry) and amniotic fluid volume per fetus for viable vs dead fetuses by total number of fetuses in utero at 51 to 53 d of gestation. Placental weight of viable fetuses decreased ($P < .05$) with increased fetal number. Amniotic fluid volume was greater ($P < .01$) for viable than for dead fetuses and decreased ($P < .01$) with increased fetal number for dead fetuses.

butions by placental and luteal tissue to maternal progesterone concentrations are unclear and may vary with stage of gestation. Removal of the CL before 175 d of gestation induces abortion, whereas its removal after 175 d produces highly variable responses (i.e., parturition within 13 to 74 d), including a normal gestation (Estergreen et al., 1967). Also, evidence exists to suggest that luteal function declines during the last trimester of gestation in cattle (Shemesh and Hansel, 1983). Thus, maternal progesterone concentrations in the first two trimesters of gestation are presumably reflective of luteal mass and function, whereas concentrations in the last trimester may include production by an extra-ovarian source(s), possibly the placenta (Conley and Ford, 1987). Because twin fetuses are maintained in cows with one CL, increased maternal concentrations of progesterone are not required for maintenance of pregnancies with multiple fetuses (Estergreen et al., 1967; Anderson et al., 1979).

The feasibility of using maternal E_1SO_4 or progesterone as a criterion to diagnose pregnancies with multiple fetuses was evaluated. Although circulating concentrations of E_1SO_4 and progesterone were significantly higher in dams with multiple fetuses, the magnitudes of the differences were insufficient to diagnose multiple fetuses reliably. Sreenan (1981) reported that circulating concentrations of total free estrogens were sufficiently higher between 216 and 223 d of gestation for cows with twins to use as a diagnostic criterion but failed to find differences in maternal progesterone concentration. Lack of specific information on the estrogen assay employed by Sreenan (1981) prevents direct comparison of the two studies.

Based on fertilization rates, calf birth weights, and calf survival rates obtained in the present study, it is anticipated that increasing ovulation rate from one to two would increase productivity in beef cattle 1.36-fold for number of live calves born and 1.08-fold in birth weight, which compares with 1.41- and 1.08-fold, respectively, for cattle with spontaneous twin ovulations (Echternkamp et al., 1990; Gregory et al., 1990b). This increased birth rate subsequently results in a 50 to 60% increase in total calf weight weaned for cows with twin ovulations (Gregory et al., 1990b) or two transferred embryos (Guerra-Martinez et al., 1990). Unfortunately, the first limiting factor to achieving this increased productivity is effective methodology to regulate (either naturally or artificially) ovulation rate and(or) number of embryos in cattle. Although triplet, quadruplet, and quintuplet calves were produced, the increased incidence of prenatal and neonatal mortality with the higher ovulation rates negated potential increases in productivity for ovulation rates above two.

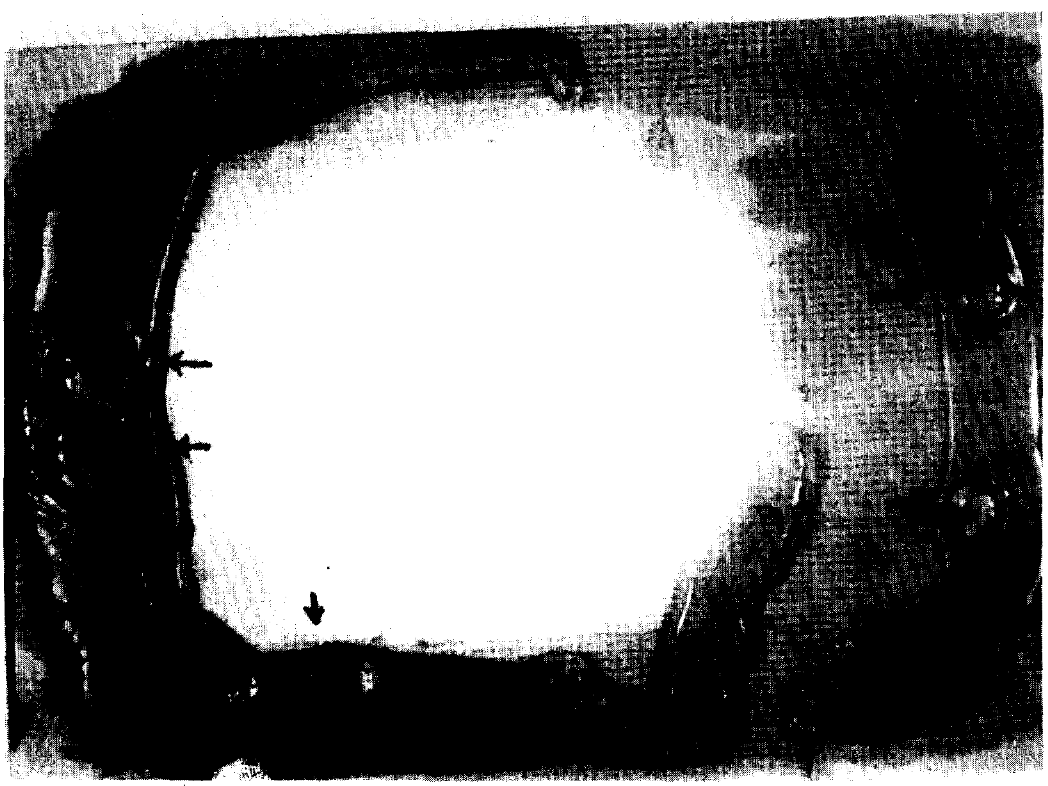

Figure 5. Fetal and placental development at 52 d of gestation in Cow 812714. The left uterine horn contained three viable fetuses and the right uterine horn contained three dead fetuses.

Furthermore, in Exp. 2 the average number of normal fetuses was two per dam when ovulation rate exceeded two. Also, the absence of degenerate fetuses at 51 to 53 d of gestation in cows with one or two CL (Exp. 2) may indicate that dams with increasing numbers of degenerate fetuses have a longer return to estrus interval. The number of triplet and quadruplet births was insufficient to assess the contribution of increased dystocia vs shorter gestational length to the greater neonatal mortality in these calves. However, Gregory et al. (1990b) reported lower survival rates for twin calves regardless of the incidence of dystocia (.73 vs .92).

Implications

Although the bovine female limits the number of conceptuses to one by releasing one ovum per estrous cycle, some cows are capable of gestating as many as five fetuses to term when multiple-ovulated with follicle-stimulating hormone. The multiple births suggest that ovulation rate is the first limiting factor to increasing beef production. However, increasing the number of fetuses in utero decreased calf birth weight and increased the incidence of fetal mortality, abortion, and dystocia; an ovulation rate greater than two produced no further increase in reproductive rate. Use of this productional opportunity for cattle awaits development of repeatable procedures to control ovulation rate or embryo numbers artificially or to increase ovulation rate naturally.

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